The University of Chicago

THE DIFFERENTIATION OF THE NASAL AREA OF THE CHICK EMBRYO IN GRAFTS

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

1937

By

SIBYL FAWCETT STREET

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Reprinted from
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THE DIFFERENTIATION OF THE NASAL AREA OF THE CHICK EMBRYO IN GRAFTS

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THREE TEXT FIGURES AND TWO PLATES (SIX FIGURES)

This study was undertaken to determine 1) how early in development the isolated prospective nasal area would show capacity for independent differentiation, 2) the approximate extent of the nose-producing area and 3) the course of outgrowth of the olfactory nerve under graft conditions. The method of chorio-allantoic grafting was used almost entirely but some grafts of olfactory placode directly into the body of a host chick were made.

Comparatively little experimental work has been done on the nose in contrast with that done on other sense organs. Hoadley ('24) found that the tip of the head of a 36-hour chick embryo grafted to the chorio-allantoic membrane would give rise to nasal tissues. Nasal tissue has not been found in grafts of the chick at pre-somite stages though these have been much studied (Rawles, '36, and others). Hiraiwa ('27) found that olfactory epithelium and nerve were present in a graft from the anterior third of a rat embryo transplanted to the chick chorio-allantoic membrane at a stage when the nasal pit just showed as a depression.

More work has been done on the Amphibia. Bell, Burr, Carpenter, Detwiler, Luna, Lewis, and May have all transplanted the olfactory placode but they were interested mainly in the effect of the ingrowing nerve on adjacent nervous tissue

and in general do not describe the differentiation of the grafted nose. They showed, however, that the presumptive olfactory epidermis was self-differentiating at least a short time before the first thickening for placode formation appeared and that the olfactory nerve, if formed near other regions of the brain than the olfactory lobes, would grow into them. Burr ('16) showed that in Amblystoma the form of the nasal capsule is dependent on the growth of the olfactory sac. The formation of the nasal placode is due to secondary induction by the forebrain (Holtfreter, '33, '36; Raven, '33), since olfactory placodes occasionally adjoin the brain of induced embryos, or grafted brain developing from pieces of the anterior end of the neural plate (Adelmann, '29 b) and double or supernumerary placodes go with doubling or abnormalities of the forebrain.

This study is part of the general analysis of the development of the chick which has been carried out by Dr. B. H. Willier and his students by means of chorio-allantoic grafting. It gives me pleasure to thank Doctor Willier here for recommending the problem to me and to express my grateful appreciation of the criticism and of the many helpful suggestions he has given me.

NORMAL DEVELOPMENT

The first morphological sign of the olfactory placode is a thickening of the ectoderm at the side of the head just anterior to the eyes, which appears in the 23-somite stage (Cohn, '03). It appears to migrate ventrally from the original lateral position because of the rapid growth of the cerebral hemispheres and sinks in to form the olfactory pit. The early stages of invagination of the pits are probably due to processes of growth within them, later deepening of the pit seems to be passive and due to the forward growth of the maxillary and nasal processes. The nerve is formed by ingrowth of the central processes, axones, of the olfactory cells

and reaches the brain about the end of the fourth day of incubation. At this stage the brain is separated from the pit only by a thin layer of mesenchyme (Disse, 1897). The beak is formed by the fusion and forward growth of the inner and outer nasal processes and the maxillary process. The ethmoid region of the trabeculae gives rise to the nasal septum which in turn gives rise to the roof and lateral walls of the nasal capsule. No floor to the capsule is formed in the chick (Gaupp, '06).

At first the olfactory pit is lined entirely with sensory epithelium but as it deepens non-sensory epithelium, the teloderm, is invaginated, forming the olfactory vestibule. The middle and superior conchae arise from the area lined with sensory epithelium the first on the fourth day, the second on the fifth to sixth day. Sensory epithelium recedes quickly from the middle concha. By the day after its formation it is limited to the upper wall and shortly thereafter is replaced entirely by indifferent epithelium. The vestibular or inferior concha has no homolog in the other classes of vertebrates. It arises on the eighth day from the lateral wall of the vestibule and from the first is covered entirely by non-sensory epithelium. The nasal or septal glands whose serous secretion serves to moisten and clean the cavities also develop on the eighth day, growing out as a solid cord of cells from the inner wall of the vestibule.

By the tenth day of incubation, which is roughly the stage of development reached by the grafts, the nasal cavities are complete. The nares open to the vestibule with its concha. This part of the passage is closed, a temporary phase during development, and lined with a stratified epithelium composed of cells similar to those of the epidermis but less closely packed and possessing a less granular, less darkly staining cytoplasm. The lumen reappears in the neighborhood of the middle concha, one passage leads to the choanae and a more dorsal one to the superior or olfactory chamber. The middle concha and the walls of that part of the nasal passage are

covered with a stratified epithelium which is thinner than that of the vestibular region. Olfactory epithelium is limited to the superior concha and the adjacent and dorsal walls of the nasal passage. The superior concha is a simple projection from the lateral wall, not coiled as are the other two.

The olfactory epithelium is a rather generalized sensory type at this stage (fig. 4). It is about 0.068 mm. high, pseudostratified, and without a basement membrane. It shows three more or less distinct regions; an outermost narrow band composed of the tips of the supporting and sensory cells in which no nuclei lie, then a broad irregular band of nuclei in which the small oval nuclei of the supporting cells and the large round olfactory nuclei can be distinguished; and third, an innermost region composed of tangled nerve fibers and branching cell processes of the supporting cells with occasional nuclei. Olfactory hairs are sporadically developed or wanting, the nuclei of the supporting and olfactory cells are not so sharply differentiated or localized as in the adult where the small dense nuclei of the supporting cells form a fairly distinct band outside the large, clear, round, olfactory cell nuclei. The flask-shaped, mucous secreting Bowman's glands present in the adult epithelium are not yet developed. This high sensory epithelium goes over fairly abruptly to the stratified epithelium of the middle region by a short stretch of a low sensory epithelium about half as high as that of the olfactory area.

The nasal glands are at this time a solid strand of cells extending from the median wall of the vestibule laterally, then dorso-posteriorly just outside the lateral wall of the nasal passage, to the orbit of the eye. The olfactory nerve leaves the olfactory chamber at the posterior dorsal angle and passes through the nasal cartilage to reach the olfactory lobe of the brain.

TIME OF DETERMINATION OF NASAL TISSUE

Nasal tissue had not been identified in grafts from presomite blastoderms. To determine the earliest stage at which

tissue isolated from the embryo would produce it a series of chorio-allantoic grafts of the tip of the head were made (fig. 1).[1] The donors varied from the late head fold to the 24-somite stage. In the older stages the epidermis and forebrain back to the optic vesicles were grafted, in younger stages the anterior half of the optic vesicles was included as well.

Eighty-four grafts were obtained in this series, the results of which are given in table 1. In the seventeen grafts from donors of the 4-somite stage or younger no nasal tissues were found although skin was present in every graft. In three out of six grafts from 5-somite embryos the scalloped, cornified skin characteristic of the vestibule differentiated, indicating that it is the first tissue of the nasal complex to acquire

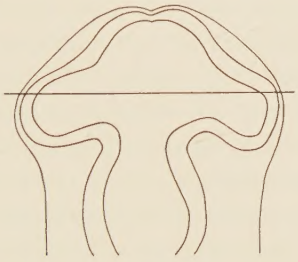


Fig. 1 Diagram of the head of a 16-somite embryo showing the location of the cut removing the tip of the head.

the capacity for independent differentiation under graft conditions. Skin in all chorio-allantoic grafts tends to become cornified, that from any region of the embryo often forming as thick a structure as the horny tip of the beak of a 10-day normal. In normal development the vestibule develops a cornified skin which differs from that of the beak in presenting a rough scalloped appearance. Each scallop is apparently the result of a series of cell divisions in a germinal cell, or a group of them, in such a way that strings of cells extending vertically from the germinal layer of the epidermis

[1] For technique see Willier ('24).

TABLE 1

Time and frequency of appearance of nasal structures in several types of grafts

Number of somites of donor	GRAFTS OF WHOLE TIP OF HEAD								GRAFTS OF PROSPECTIVE NASAL SKIN ALONE				GRAFTS OF PROSPECTIVE NASAL SKIN + FOREBRAIN			GRAFTS OF PROSPECTIVE NASAL SKIN + MEDIAN FOREBRAIN		
	Number of cases	Olfactory epithelium	Low sensory epithelium	Vestibular skin	Vestibular conch	Middle conch	Nasal gland	No skin	Number of cases	Olfactory epithelium	Low sensory epithelium	Nose anatomy	Number of cases	Olfactory epithelium	Low sensory epithelium	Number of cases	Olfactory epithelium	Low sensory epithelium
HF[1]	6																	
1	3																	
2	4																	
3	1		1															
4	3																	
5	6		1	3	??			1										
6	7			1	?			1										
7	5		1	1	?													
8	6							2										
9	5	1	1	2	?													
10	3							1										
11	4																	
12	7	3		4	3	1	1											
13	4	2		2									1	?				
14	4	2		2	2	2	?						2	2				
15	4	2		2		1							2		1	1		
16	0												1	1				
17	1	1		1		1							1	1				
18	2	1		2	?	1			1				3	1		1		
19	1												5	3	1			
20	2	2		2		1	?						1					
21	3	1		2	1	2			6		3	2	2	2		1	1	
22	0								3				2	1	1	4	3	
23	2	2		2	2	2	1		7		3	4				5	4	1
24	1	1		1	1	?			9	3	3	4	1			2		
25									5	?	2	3				1	1	
26									6	2?	1	4				3	2	
27									1	1		1				2	2	
28–48									19	13	5	17				3	3	
Total	84								57				21			23		

[1] Head fold stage.

? Questionable differentiation.

are formed (fig. 5). The differentiation of this vestibular skin in the grafts occurs several days sooner than in a normal embryo. It is found in grafts whose total age is as little as 9½ days while in a 12-day normal the vestibular epithelium is still of the simple stratified type. It is often the only trace of nasal tissue found in grafts. In these grafts from young donors the scalloping generally is not so sharp or so extensive as in the older ones. It occurs in conjunction with irregular cartilages which perhaps represent those of the vestibule. In one case it was traced to a vesicle of low sensory epithelium of the type which occurs in the normal where olfactory grades over to stratified. This cannot be distinguished from any other generalized embryonic sensory epithelium such as the sensory layer of the retina or the brain wall in an early stage of differentiation. However, it may represent the first stage of differentiation of olfactory epithelium, as it also resembles the epithelium of the placode of the 26-somite embryo, the stage just before invagination begins. Six and 7-somite donors also gave cases of the type of differentiation just described, namely, vestibule-like areas leading in one case to a small sac of stratified epithelium such as lines the middle chamber, in the other ending blindly but close to a vesicle of low sensory epithelium.

Olfactory epithelium first developed in a graft from a 9-somite embryo. It is quite typical in appearance, with olfactory nuclei clearly differentiated from those of the supporting cells. Only one nasal area developed in this graft. The sensory epithelium connects by a short stretch of stratified epithelium with a quite extensive area of vestibular skin and there are adjacent irregular cartilages (fig. 7). By the 9-somite stage then, all the epithelia of the nasal passage are able to differentiate in the grafts.

At the 12-somite stage the frequency of appearance of olfactory tissues suddenly rises to about 50% and organogenesis becomes more normal. In one of the grafts from a donor of this age, in which only one placode differentiated, development was as normal as in any comparable case from

an older embryo (fig. 6). There is a vestibular area leading to a middle chamber with an abnormal but recognizable concha which in turn leads to the superior chamber, lined with olfactory epithelium but having no concha. The cartilage capsule is fairly normal and the olfactory nerve goes to the brain through a foramen at its (probable) dorso-posterior angle. Nasal gland develops in normal relation to the vestibule.

The appearance of such a well-developed organ at this stage, when in fifty-three grafts from younger donors only abortive vestibule-like structures and one case of olfactory epithelium had been obtained, suggests that some change occurs in the embryo at this time. This change might involve either a specific determination of the cells for nose production or the attainment of anatomical relationships more favorable to the development of the organ under graft conditions. The latter possibility is the more probable since in the earlier grafts, as we have seen, all the epithelia were capable of differentiation. The tip of the head is changing rapidly in the 9- to 12-somite stages; the suture cerebralis anterior is closing, the anterior wall of the forebrain vesicle bulges out and mesenchyme begins to appear in the prospective nasal area. The appearance of the mesenchyme is probably the critical factor. In Amphibia the nasal capsule is formed by mesectoderm which migrates forward from the neural crest over the mid-brain (Landacre, '21; Stone, '26, '29). In the chick the source of the material for the capsule is not definitely known but probably both neural crest and prechordal plate contribute to it. Conditions in the graft may be unfavorable for the migration of the mesenchyme, resulting in a mechanical check on the invagination and development of the placodes in grafts from donors younger than this stage.

There is no further increase in the frequency of nose production until after the primordium appears but the differentiation of the middle and vestibular conchae becomes more exact.

LOCALIZATION OF THE NOSE-FORMING AREA

In the urodeles (Adelmann, '29 a, '29 b) and in the chick (Clarke, '36) the potential eye-producing area has been shown to occupy both median and lateral regions at a certain level of the blastoderm. In the chick Clarke found that even at the 8-somite stage, when the optic vesicles are already formed, the brain floor at the eye level will produce eye when grafted. The mapping of the organ forming areas of the blastoderm has shown that several other organs which are later limited to the lateral areas originally consist in a single harmonic equipotential field. No such work has been done on the extent of the nose-forming area but it is known that in the frog the primitive placodal thickening arises as a continuous band just outside the neural plate and later subdivides into olfactory, hypophyseal and other placodes (Knouff, '35). At a stage when the olfactory placodes have just been formed the adjacent tissue can readily regenerate an excised placode (Bell, '07). Also in tissue cultures, of whole chick embryos Waddington and Cohen ('36) found that when the right half of the head at the forebrain level is removed at the 15-somite stage, repair of the forebrain occurs and a nasal placode is induced in the regenerated epidermis. Thus it seems possible that the nose-producing areas might arise as a single field which later subdivides.

To determine whether the potential nose-producing area extended to the median line, or was present only laterally, a series of grafts were made in which the tip of the head was divided into right, left, and median pieces which were grafted separately. In young donors the grafted area extended slightly into the optic region.

The donors varied in age from 8 to 24 somites but the majority of cases were from donors between the 13- and 20-somite stages. The width of the median piece was measured with an ocular micrometer when the cuts were made; it varied from approximately 0.19 mm. to 1.12 mm. (fig. 2). The groove marking the closure of the neural folds is still visible in these embryos so that it was an easy matter

to be sure of the location of the median line. The median piece was generally cut so that it included equal amounts of material from either side of the median line.

Eighty-nine grafts were obtained in this series. Thirty-two from right pieces, thirty-five from median and twenty-one from left. The results are given in table 2. The data for the median pieces are given in detail but those for the right and left pieces are summarized since they showed no correlation between the occurrence of olfactory tissues and the width of the median piece.

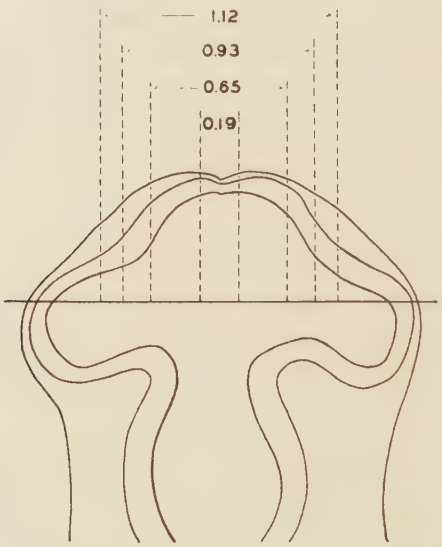


Fig. 2 Diagram of the head of a 16-somite embryo showing the range in width of the isolated median piece. No nasal tissue was found in grafts from pieces narrower than 0.65. Measurements in millimeters.

Olfactory epithelium could not be positively identified in any graft from a median piece. In the one possible case marked with a question mark in the table the median piece was wide enough (0.93 mm. donor 13 somites) so that some of any laterally located nose producing area would have been included. The lateral pieces from this donor were grafted to a host that died before the grafts were recoverd. Low-sensory epithelium and what I have called in the table nose

anatomy, meaning an arrangement of epithelia and cartilages characteristic of the nasal passages, appear only in grafts from median pieces at least 0.93 mm. wide. The vestibular skin producing area extends further toward the midline, as would be expected since it must lie around the olfactory area. This skin appeared in half of the grafts cut 0.65 mm. wide or wider and does not occur in grafts from pieces cut narrower than this.

TABLE 2

Localization of the nose-forming area

DATA FOR MEDIAN PIECES

Width of median piece	Number of cases	Olfactory epithelium	Low sensory epithelium	Vestibular skin	Nose anatomy	No skin
mm.						
0.19	1					
0.28	1					
0.37	9					5
0.46	5					2
0.56	6					3
0.65	4			2	?	
0.74	6			2		
0.84						
0.93	4	?	2	3	1?	
1.02	1					
1.12						
Totals						
Median pieces	35		2	7	1	10
Right pieces	32	4		5	5	10
Left pieces	21	4	2	3	5	4

? Questionable differentiation.

However, nasal tissue developed very infrequently in the grafts from lateral pieces, olfactory epithelium appearing in only eight out of fifty-three grafts (15%). This is a much lower percentage than in the previous series where the whole tip of the head was grafted. In that series in grafts from donors of 9 somites or older the frequency was 41%, eighteen out of forty-three grafts showing olfactory epithelium. Of course when the whole tip of the head is grafted two prospective areas are included and in eight of the eighteen positive

cases both areas developed. If the percentage of frequency is calculated on this basis, then eighty-six prospective areas were grafted of which twenty-six developed (30%).

In the series of grafts under discussion skin did not appear at all in twenty-four of the eighty-nine grafts obtained, and in several others the only trace of it was a small tube or a rod of cornified cells in characteristic arrangement. In a few, only the presence of a lens indicated that skin must have persisted for a time after the graft was made. In the grafts of the whole tip of the head of donors of comparable age, skin was present in every case. Since in each series about 30% of the grafts made were recovered it would seem that no general factor affecting the viability of the embryos was involved. It is merely that small pieces of head skin do not develop well in these grafts. This is shown more strikingly in the next series of grafts in which skin alone was grafted. The failure of the skin to persist in the grafts, I believe, accounts for the low frequency of olfactory tissues in this series. Olfactory epithelium developed in 21% of the lateral grafts in which skin persisted (eight out of thirty-nine cases). In comparison its non-appearance in the twenty-five grafts of median pieces in which skin persisted is barely significant statistically, therefore, it seems likely that at the stage studied the capacity to differentiate into olfactory epithelium in chorio-allantoic grafts is limited to the lateral areas.

GRAFTS OF PROSPECTIVE OLFACTORY EPIDERMIS

A series of grafts of prospective olfactory epidermis alone were made to see if it could differentiate into olfactory tissue in the absence of brain. The area grafted is indicated in figure 3. The epidermis was freed by means of fine glass needles over a semicircular area extending in front and in back of the eye. In the older embryos only the anterior part was included. In general, I tried to make the piece as large as possible because this tissue incorporates very poorly in the younger stages. Indeed, although there were 146 cases where the prospective olfactory epidermis from donors of 6 to 22

somites was grafted to hosts which lived for 9 days, only ten grafts were recovered. No grafts were recovered where the donor was younger than 18 somites. After the 22-somite stage such grafts develop frequently.

The data for these grafts are given in table 1. Olfactory epithelium did not appear in grafts from donors younger than 24 somites, a stage at which the placode is already present as a thickened plate anterior to the eyes. However, other nasal structures differentiated. In one of the grafts from a 21-somite donor there was even a middle chamber with a concha and an area of low sensory epithelium in addition to a vestibule.

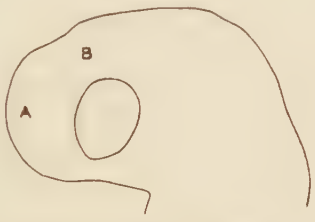


Fig. 3 Diagram of the lateral view of the head of a 21-somite embryo. The dotted line outlines the area of face skin removed for grafting. In older embryos area B was not included.

I had noticed when isolating the tissue for these grafts that as soon as the epidermis was freed from the brain it shrank to about half its size. The shrinkage was greater the younger the donor, and must be due to the release of the tension caused by the rapid growth of the forebrain. To see if this shrinkage alone was enough to inhibit the development of nasal tissue another series of grafts was made. The whole tip of the head was again grafted but before it was cut off from the embryo a cut was made through the epidermis only, in front of the eye along the posterior and ventral borders of the prospective nasal area. In young donors up to the 14- or 15-somite stage this cut was sufficient to cause the skin dorsal to it to shrink to at least two-thirds its former size. In older donors to produce as great a shrinkage the flap had to be freed along the anterior edge as well.

Few grafts were made in this series but sufficient to show that nasal tissue would develop under these conditions in grafts from donors as young as 14 somites. Although the prospective olfactory tissue overlay a more dorsal region of the forebrain than would normally be the case and did not undergo the usual stretching, nasal tissue developed as normally as when the unoperated tip of the head was grafted. The olfactory nerve grew into the brain tissue which was presnt. In table 1 the results of these grafts are listed as "grafts of prospective nasal skin and non-olfactory forebrain."

A variation of this type of graft was done on embryos of 18 somites or older. The skin in front of the eyes on each side was freed from the brain up to the median dorsal line and the brain cut off except for a small portion which served to hold the skin of the two sides together. The graft thus consisted of most of the skin of the head anterior to the eyes with a small amount of brain at the dorsal median line. The results for this series also are given in table 1 as "grafts of prospective nasal skin and median forebrain." Olfactory epithelium was present in eight grafts out of the twelve obtained from embryos of 23 somites or younger. It did not appear in grafts of the same area of skin in which the bit of brain was not included. It is impossible to say definitely whether this difference in behavior is due to induction of nose by the brain present in the one type of graft or merely to the fact that the piece of tissue involved was larger and so incorporated and grew better on the membrane. Since the frequency of development of olfactory epithelium in these last two types of grafts is the same as that for grafts of the whole tip of the head from donors of the same ages, about 50%, it would seem that the factor of size of piece is of the greater importance.

In several of these grafts the nasal areas of the two sides came together so that a fairly normal nose was formed. The nasal septum was single though it might have been expected that mechanical separation would result in doubling.

Some grafts were made of the nasal epidermis alone in later stages of development. The nasal pit with the surrounding epidermis and subjacent mesoderm was freed from the forebrain and grafted. Nasal tissues were produced and the organ formed was similar to that described in grafts from younger stages, except for a continued improvement in organogenesis. In a graft from a 32-somite donor a middle concha as perfect as the one shown in figure 8 first developed. A very abnormal superior concha was formed in one graft from a 24-somite donor, in a graft from a 36-somite donor a well-differentiated one first appeared. In a few cases the greater part of the mesoderm was removed before the tissue was grafted. The mesoderm cells lying directly beneath the ectoderm and clinging closely to it were left. In these grafts also a quite complete cartilage capsule was formed and conchae developed.

The stratified epithelium of the middle chamber and concha generally resembles that of the normal of the same age. But in some of these grafts from the oldest donors used (30 to 40 somites) its differentiation was accelerated and patches of ciliated respiratory epithelium were found.

THE OLFACTORY NERVE IN CHORIO-ALLANTOIC GRAFTS

Whenever the sensory epithelium was well differentiated nerve fibers were produced and generally a nerve was formed. These contained irregularly scattered ganglion cells lying on the surface of a strand of fibers or buried among them, as occurs in the normal nerve. When the presumptive nasal area and forebrain were not separated but left in normal relation to one another when grafted, the nerve was always found to have reached the brain, although the two organs might have become widely separated in the course of development. (There is one case in which it is possible the nerve did not reach the brain but I believe this appearance is due only to poor histological technique in handling the material.) In many grafts, because of the failure of the cartilage capsule to develop, the olfactory epithelium was separated from the brain only by a thin layer of mesenchyme or even invaded

the brain cavity. In these cases frequently no distinct nerve bundles formed, the nerve fibers presumably reaching the brain individually. I say presumably, because there is no way of distinguishing between olfactory nerve fibers and fibers which might be going from the brain to the epithelium. When two placodes develop in the same graft their nerves may join and enter the brain together. No olfactory lobe of the brain was found, probably because of the invariably disorganized, blood-filled condition of brain tissue in these grafts.

When the olfactory placode or presumptive olfactory area alone was grafted, nerve fibers were formed but showed no directed growth. Most frequently a large ball of nerve fibers was formed near the olfactory epithelium, as if the fibers had grown around on each other, but no nerve left the cartilage capsule. When a nerve bundle did leave the olfactory area it ran along the inside or outside of the cartilage capsule for a short distance or went a short way into the graft mesenchyme before disappearing. I have found none leaving the graft area to enter the chorio-allantoic membrane, and they show no consistent tendency to grow toward other structures in the graft when such are present.

In grafts from the type of isolation described in a previous section, where a bit of median dorsal brain was included with the two olfactory areas, the developed nerve showed some variety of behavior. In seven out of fourteen cases where an olfactory nerve was produced some or all of it reached the brain tissue and joined with it, although the presumptive olfactory lobe material of the brain was not present. In the remaining seven cases, although brain tissue was well developed the nerve did not grow to it, even though to all appearances there were no mechanical hindrances to check such growth.

In grafts from a somewhat similar operation done on younger embryos, where the presumptive nasal epidermis was loosened and allowed to shrink until it overlay more dorsal brain than normal, the nerves reached brain tissue in every

case in which they were formed except one. There were eleven cases in which the olfactory nerve developed. The exceptional case was one in which the only certain trace of brain tissue was a bit of pigment layer of the retina. Only one placode developed and that very incompletely. In two of the other cases both placodes developed but the nerve from only one reached brain tissue.

GRAFTS INTO THE BODY OF A HOST

Transplants of olfactory pits were made directly into the body of the embryo to obtain material to compare with the chorio-allantoic grafts in regard to general differentiation and more especially the outgrowth of the nerve. The technique used was developed by Hamburger ('33) for the transplantation of chick limb buds. A large window was made in the host egg with a small hack saw blade and the egg was mounted under a binocular microscope, then with a fine glass needle a slit was made in the body wall just anterior to the leg bud and the tissue to be grafted pushed into the slit. The body wall is elastic enough to hold the tissue in place. The tissue grafted was from donors of 30 to 40 somites and included the skin and mesenchyme surrounding the pits as well as the pit itself. Where both pits from a donor were grafted together they were left connected by a band of skin and placed in the slit as a long strip of tissue, not folded together. The shell window was sealed back into place with paraffin and the egg allowed to develop for from 5 to 7 days. The embryos were then preserved in Bouin's fluid. Nineteen of these that showed a growth near the right leg were sectioned at 8 μ and studied. Two proved necrotic and were discarded.

Nasal tissue was present in all but three of the cases examined and in all of these well-formed olfactory epithelium and nerve had developed (fourteen cases). The graft generally grew out as a small papilla between the leg and wing of the host chick. Its base was usually covered with feather germs which, taken with the finger-like processes often present at its tip, made it appear remarkably like a limb bud. The

internal anatomy showed no indications of any such structure, however, the appearance of fingers being due probably to the growth of the median and lateral nasal processes. In all but one case the graft was innervated by one or more branches from the lateral rami of the spinal nerves of the region. Since these grafts generally were located just anterior to the leg, the spinal ganglia most frequently involved were the twentieth to the twenty-third; the twenty-third is the first ganglion of the leg plexus. These host nerves seemed invariably to penetrate the graft tissue accompanying the blood vessels. Innervation occurred irrespective of the presence or absence of nervous tissue in the graft.

The development of nasal tissue in the transplants was quite comparable to that found in the chorio-allantoic grafts so I will discuss only the few points of difference between them. The graft tissues never appeared cramped for space as is sometimes the case in the chorio-allantoic grafts where the epithelia are often folded instead of smooth. There was no acceleration of differentiation of the vestibular or middle chamber epithelia, the stage of development reached being comparable to the normal or slightly retarded. Morphological development in one case, where both placodes were grafted and developed in normal relation to one another, showed less distortion than did the best of the chorio-allantoic grafts (fig. 9). The nares were generally located toward the tip of the papilla in normal relation to the closed vestibular passages, while in the chorio-allantoic grafts they were buried in the mesenchyme of the membrane and often enlarged or not recognizable.

Olfactory epithelium differentiated well and produced large masses of nerve. There were nine cases where a single placode developed, five where two developed, so that in all nineteen nerves were formed. Six of these did not leave the immediate neighborhood of the epithelium, nine grew out into the mesoderm a short distance and small branches of three of these anastomosed with branches of the spinal nerves which were nearby. The other cases were somewhat exceptional and will be discussed in more detail.

In two of these cases there was direct fusion of the olfactory nerve with a large spinal nerve entering the graft. In the first of these the fusion occurred with two branches of the same spinal nerve at the point where the olfactory nerve emerged from the cartilaginous capsule at the base of the graft papilla. From the clump of nerve fibers thus formed a nerve goes forward in the papilla and anastomoses with another branch of the same spinal nerve; a group of the spinal nerve fibers could be traced back into the capsule as one area of the apparent olfactory nerve, across the capsule and out along a blood vessel till it broke up near the epidermis of the tip of the papilla as does any cutaneous nerve. As far as could be determined olfactory fibers did not accompany it. A third nerve which was probably composed of olfactory fibers ran anteriorly in the ventral body wall of the host for some distance, first with a muscle bundle then with a blood vessel becoming smaller as it went. It finally disappeared in the mesenchyme near the tip of a rib.

The second of the cases of this type was one in which a small amount of brain tissue must have been included with the two placodes when they were grafted. Histological study of the graft after six days growth showed brain tissue present consisting of a very small tubule about 50 μ long embedded in a mass of nerve fibers, some olfactory and some from the host. The two olfactory nerves come together around the brain tubule and branches of two spinal ganglia run to it. Several small nerves leave this mass of fibers to go out into the papilla where they either break up near the epidermis like cutaneous nerves or disappear in the mesenchyme as olfactory nerves generally do in grafts. This case suggests an amazing degree of attraction by the brain tissue for the nerves but I believe this would be a false conclusion to draw from it. The host nerves entering these grafts showed a distinct tendency to enter along the sides and grow toward the tip staying not far inside the dermis. The brain tissue lay directly in their customary path. Also, in other cases when two placodes developed in the same graft, the olfactory nerves joined each

other at about the same point in relation to the whole as in this case, though there was no brain tissue present in them.

The third case is the only one where without a doubt the olfactory nerve grew into the host from the graft. The left placode of a 36-somite donor was grafted as usual and allowed to grow for about 5 days. Histological examination showed typical nasal tissues developed. The nerve leaves the cartilaginous capsule with a blood vessel and goes straight into the body wall of the host running forward and ventrally for about 800 μ. It stops abruptly in the mesenchyme near the sternal cartilage. The nerve, which contains many scattered ganglion cells, does not branch or get smaller; it remains the same size throughout its course. It follows the same general route as the blood vessel with which it left the olfactory capsule but does not stay in contact with it after leaving the graft papilla.

THE INFLUENCE OF THE MESODERM

Some attempt was made to determine whether the cartilaginous skeleton of the nasal capsule would form in the absence of the proper epithelium. Embryos of 30 to 45 somites were used. The skin in front of the eyes was isolated as in the previous series of experiments but the olfactory pit was shelled out and grafted separately. It was found that with a little care, the layer of mesoderm lying between the pit and the brain could be left unbroken but some mesoderm cells always clung to the base of the sensory epithelium and were grafted with it. Sixteen grafts of the pit alone were recovered and forty-six of the skin and mesoderm adjacent to the pit. The result showed that at the stage used, when the pit is lined with sensory epithelium only, the nose field is either still a partially equipotential system or that the parts possess considerable powers of regeneration.

The pit grafted alone generally developed only into a small epithelial sac with a couple of small cartilage bars but in two cases it formed a small but relatively complete olfactory passage. The best one consisted of a small loop of vestibular

skin leading to a midde chamber with a typical cartilage capsule and a concha and an extensive area of olfactory epithelium which had produced a nerve, the other had a larger vestibular area but no olfactory epithelium. This result accords with the prospective potency of the area grafted. The presence of vestibular skin is accounted for by the fact that there is no sharp line of demarcation between the sensory and non-sensory epithelia at the stage when the isolation was made, so that in trying to remove all the sensory epithelium some of the other was included. There was a small amount of vestibular skin in six of the sixteen grafts of this series. That the small amount of mesoderm included could form so complete a capsule is surprising.

The grafts of the pit alone suggest a strictly determined mosaic pattern but the grafts of the skin and mesoderm adjacent to the pit do not bear this out. In most of these the only nasal structure present is the vestibule, but in four grafts well-developed middle chambers with capsules and conchae lined with squamous and low-sensory or respiratory epithelia were found. In two other of the forty-six grafts of this series a small amount of disorganized olfactory epithelium was present but my operating notes show that in one of these a small piece of the pit was left in and the other was from a young donor with a shallow pit, a stage when it is difficult to be sure all the sensory epithelium is removed. Therefore it seems safe to conclude that the non-sensory epithelium of the vestibule cannot regenerate the sensory area but can regenerate or replace the respiratory epithelium of the middle chamber. As far as the mesoderm is concerned it is clear that either the layer immediately adjacent to the epithelium or that lying somewhat deeper in, can form the olfactory capsule.

When the epithelia do not develop, no recognizable capsule is formed though slender curved cartilage bars resembling those making up the capsule appear. Such bars are not found in control grafts of skin and mesoderm from in back of the optic cup. Similarly, when the epithelia of the olfactory

passage are present but the cartilage is poorly developed or absent, the shape of the chambers is not normal. This indicates that both the epithelia and the cartilage can undergo histological differentiation independently of one another but that nevertheless for good morphological differentiation they must interact. It is probable that the formation of conchae depends on growth factors in the mesodermal component since, in the case of the middle concha at least, the removal of its normal epithelium does not prevent its development. Also conchae in the grafts are invariably supported by cartilage bars and in many grafts the cartilage of the middle concha is more coiled than is its covering epithelium. In one case (27-36), a graft from a 15-somite donor, an apparent middle concha is covered with cornified skin instead of the normal low stratified epithelium. The importance of the mesoderm in the development of the nose was also suggested by the fact, pointed oue previously, that the prospective nasal area when isolated before the appearance of head mesoderm dfferentiates infrequently.

DISCUSSION

Determination of the nasal complex. It would seem from this study that the nasal complex of the chick is composed of parts which are able to differentiate more or less independently of one another. In the normal course of development there are two distinct stages in the formation of the nasal passages, first the formation of the olfactory pit which is due to processes of growth and differentiation within the placode, second the deeping of the pit and the formation of the choanae which is due to the growth of the mesodermal processes surrounding the pit. Graft conditions sometimes separate these stages. The first stage alone may occur so that the only nasal structure formed may be a vesicle of olfactory epithelium or a middle chamber. On the other hand the first stage may not occur but the second growth period go on to some extent resulting in an isolated and distorted vestibule-like structure. Whether the middle chamber can develop as does the vestibule, when the olfactory placode does

not differentiate, was not determined since the possibility of the placode having formed and later retrogressed is not excluded. It may be well formed in a graft in which no olfactory epithelium is present and it can form in the grafts after excision of the sensory epithelium of the pit. The olfactory area of the nasal passage is generally reduced in respect to the respiratory and vestibular regions. Frequently olfactory epithelium is limited to a patch near the base of the middle concha or may be differentiated no farther than the low-sensory stage characteristic of the just invaginated placode, also the superior concha develops only in grafts from the oldest donors in which the whole nasal complex is present. This reduction may be due to mechanical interference with the growth of the pit in the graft, which might affect most the apex of the pit where the olfactory epithelium is located. Except for the superior concha then, each part of the nasal passage can differentiate, or at least persist in a graft, independently of any other part.

In studying the localization of organ-forming areas in the chick blastoderm at the head-process stage, Rawles ('36) found that generalized sensory epithelia, somewhat resembling the type here called 'low sensory,' appeared frequently in grafts from the brain-forming level and not in grafts from other areas. This level includes most of the head process and some of the blastoderm immediately anterior to it. Doctor Rawles has kindly permitted me to examine some of her slides to determine whether these could represent olfactory epithelia inhibited at an early stage of differentiation. I found that similar epithelia occurred in my own grafts of head fold and 1- to 3-somite stages in which most of the head-forming area was grafted. These do not occur in grafts of older donors which included only the tip of the head so they cannot be olfactory epithelia.

The nose is markedly later than the other sense organs in acquiring the capacity for independent differentiation. The eye, ear, lens and also the hypophysis which while not a sense organ arises as an ectodermal placode, all appear in grafts of

pre-somite blastoderms but the nose does not differentiate in grafts from donors younger than 12 somites. Some difference would be expected since the nasal placode does not appear until the 23-somite stage, some time after these other organs are laid down, but the difference is surprisingly large in the case of the lens and hypophysis since their placodes first appear between the 15- and 20-somite stages.

The nose differs also from these and other organs in being already strictly localized when it first becomes self-differentiating. This is shown by the fact that a median piece from the tip of the head does not form nose when isolated and by the failure of more than one nasal area ever to develop in a graft of one prospective nasal area, or two in a graft in which both were included. In contrast to this the eye, ear, forebrain and many other organ-forming areas are harmonic, equipotential systems lying as bands across the blastoderm at certain levels when they acquire the capacity to differentiate in chorio-allantoic grafts. In the frog the presumptive nasal anlage when isolated at the neurula stage and explanted gives nose only when some of the medullary fold is included; the forebrain which develops from the medullary tissue induces the nose formation (Mangold, '33). The nasal pits differentiate readily in exogastrulae, chimaeras and implantations, as many as seven sometimes appearing in one area. To determine whether forebrain also induces nose in the chick it would be necessary to operate on the embryo in the egg since isolated head-skin of young embryos survives so seldom on the chorio-allantic membrane. That forebrain is the inducing agent is probable since the effect of cyclopia on the nose is the same in the chick as in the Amphibia, where it has been shown that the approximation of the nasal pits is correlated with the degree of bilaterality of the telencephalon (Adelmann, '34). If this is so, the induction must necessarily occur before the mesoderm separates the forebrain and skin from one another. At this stage it might be expected that the potential nose-forming area would be less strictly localized that it was found to be at the later stage, when it was first

capable of differentiating frequently in the grafts. Waddington and Cohen's experiments mentioned above are also interesting in this connection since they show that the nose field is quite labile when it is a question of regeneration rather than of differentiation of isolated parts.

The capacity for differentiation of the isolated nose-forming area increases with the age of the donor embryo. Before the 12-somite stage good histological differentiation of the nasal epithelia occurs, but very infrequently, and the capacity for organogenesis is slight. At that stage the frequency of nose production abruptly rises to a level maintained until the appearance of the promordium and organogenesis becomes markedly more normal. This change is probably correlated with the forward migration of the head mesoderm. The frequency of nose formation and the type of organogenesis in grafts from donors of 12 to 23 somites is the same in all the types of grafts studied except those of isolated prospective nasal skin. In these, olfactory epithelium does not differentiate beyond the low-sensory stage and the frequency of production of other nasal tissues is reduced. From 23 to 35 somites there is a rise in nose frequency, an increase in the size of the nose produced and a continued improvement in organogenesis, especially marked by the appearance of the superior concha.

It is interesting that both in the grafts of the tip of the head and of the isolated prospective nasal skin the differentiation of olfactory epithelium first occurs after that of the other nasal tissues, although in normal development it is the first to differentiate.

Any consideration of the time at which 'determination' occurs in the nose-forming area is entirely theoretical since the area shows an increasing capacity for differentiation as the embryo develops. As was pointed out by Willier ('33) in his study of the gonad-forming area, a progressive increase in the developmental potencies of an organ-forming area can mean either that the area is specifically determined from the beginning but that the mechanical conditions of the graft

interfere with morphogenesis, resulting in abnormalities and incomplete differentiation or, as he considered more probable, that there is a progressive epigenetic change going on within the area, of which the increasing frequency and specificity of organ formation is an index, the completely 'determined' condition, therefore, being the result of a chain of processes. I believe the delayed differentiation of the olfactory epithelium relative to the rest of the nose is the result of inhibition by the conditions of growth in a graft rather than an indication of a later time of determination. Otherwise the progressively greater developmental potencies of the nose-forming area probably indicate such a progressive advance in the organization of the area.

Olfactory nerve outgrowth. There has been much discussion of the stimuli which cause nerve axones to grow out and which direct their growth. Actively growing or differentiating regions in general seem to attract nerves (Detwiler, '36). Coghill ('24) pointed out that at the time of the ingrowth of the olfactory nerve in Amblystoma the cerebrum is undergoing an accelerated differentiation. An isolated olfactory placode of the chick can produce a nerve and, in cases where the morphology of the nasal passages is like the normal, this nerve may leave through a foramen which is located in the correct normal position. This means that the small bundles of nerve fibers formed near the epithelium anastomosed to form the olfactory nerve and grew away from the apex of the pit. Under normal conditions growth in such a direction would bring it to the brain, but the presence of brain at the time of the outgrowth of the axones was not necessary either to stimulate the growth or to direct it. The directed outgrowth of the nerve therefore cannot be due to a continuously acting electrical or chemical stimulus emanating from the brain but it might be that a mechanical factor is the controlling one. The theory that the orientation of the micellae of the colloidal substrate guides the nerves seems applicable (Weiss, '34). In this case the micellar orientation of the embryonic ground substance could be due to the activity

of the rapidly growing brain before isolation. Under favorable graft conditions such an orientation might persist.

Attraction of growing nerve fibers by brain tissue is suggested by some of the other series of experiments. When the transplant included brain underlying the placode the nerve almost invariably grew to it even though the presumptive olfactory lobe material was not present. When only a small piece of brain was included in the graft, not directly underlying the placode, the nerve grew to it in half of the cases. This indicates some sort of attraction by the brain tissue for the nerve. But this attraction is very irregular in its action, since in some cases where the nerve failed to reach the brain the two were quite close together in the graft and no apparent mechanical hindrance was present. There is also the fact, which should not be overlooked, that brain tissue in the grafts is loosely organized and therefore can be easily penetrated by an ingrowing nerve. Whether this alone could account for the apparent attraction of the brain tissue is a question which cannot be decided by the methods used here. In the Amphibia the fact that the nerve from a grafted olfactory placode will join many different parts of the nervous system, such as brain, ganglia or other nerves, has been shown by several workers. But here too the results are very variable, the aberrant nerve fibers ending blindly in the mesoderm almost as often as they join nervous tissue (May, '27; Stone, '24; Bell, '07; Burr, '24).

In the grafts made into the body wall of a host embryo, a distinct difference in the mode of outgrowth of the grafted olfactory nerve as compared with that of the host nerves innervating the graft papilla was noted. The host nerves invariably followed blood vessels and broke up into finer and finer branches near the skin while the olfactory nerves very seldom followed blood vessels and either ended abruptly or faded away seemingly by loss or stoppage of individual nerve fibers. Apparently axones from different types of neurones respond differently to the same environmental stimuli.

There remains to be discussed the acceleration of differentiation of vestibular skin which occurred in the chorio-allantoic grafts. The youngest grafts in which this skin developed are about 9½ days in total age, a 12-day normal embryo shows no indication of the formation of the scallops characteristic of vestibular skin although the epithelium of the vestibule is somewhat more cornified than that of a 10-day embryo. I have examined no stages between this and the 21-day when it is fully differentiated and so cannot determine exactly the degree of acceleration. However, it is a matter of at least 3 or 4 days. Transplants into a host embryo of the same age as the donor show no trace of it but all except three of these were killed when younger than the chorio-allantoic grafts. These three were grafts in good condition and as old as chorio-allantoic grafts which show it well differentiated but in them the vestibular area had not even commenced cornification. Skin and feathers also show accelerated differentiation when grown on the chorio-allantoic membrane (Butler, '35) and thyroid (Rudnick, '32) shows it occasionally. It might be true of many tissues but remain unobserved except in the few which show a definite change in structure at about the age reached by the grafts. It could be the result of some physical factor such as a better supply of oxygen in the blood stream of the older embryos or of chemical differences in the composition of the blood.

SUMMARY

1. The prospective nasal areas of chick embryos of varying age were isolated and their potencies tested by grafting to the chorio-allantoic membrane. The skin was sometimes left in normal relationship with the underlying brain, sometimes freed from it or grafted with non-olfactory brain.

2. It was found that the parts of the chick nose, such as vestibule, middle concha and olfactory area with their characteristic epithelia, were able to differentiate independently of one another in chorio-allantoic grafts.

3. A series of grafts of the tip of the head of embryos of late head fold to 24-somite stages and of isolated prospective nasal skin of embryos older than 24 somites show that the ability to differentiate into these parts is progressively acquired. The scalloped, cornified skin characteristic of the vestibule first appeared in grafts from donors of 5 somites, olfactory epithelium from those of 9 somites. At 12 somites the vestibular and middle conchae developed while the superior concha appeared only in grafts of older embryos of 35 somites.

4. At the 12-somite stage the frequency of the differentiation of nasal tissues rises from 13% to 50% and there is simultaneously a great advance in morphogenesis. This change is probably correlated with the appearance of mesoderm in the nose-forming area.

5. An attempt was made to determine the medio-lateral extent of the nose-forming area by grafting separately right, left and median pieces of the tip of the head of embryos from 8 to 24 somites. It was found that small pieces of skin from this area develop poorly so that nose-frequency was low even in grafts of the lateral pieces. The results indicate, however, that the potential nasal areas are already laterally located when the capacity to differentiate fairly frequently on the chorio-allantoic membrane is acquired.

6. The skin of the tip of the head of embryos younger than 25 somites isolated from the brain and grafted generally failed to survive. It did not give rise to olfactory epithelium unless the olfactory placode was morphologically present at the time of isolation. Results from other types of isolations indicate that probably this epithelium does become self-differentiating in the absence of brain before the appearance of the placode, but that the low survival value of skin at this stage prevents its demonstration.

7. The sensory epithelium of the olfactory pit of embryos of 30 to 40 somites was shelled out with a very thin layer of mesoderm left adhering to it and grafted separately from the adjacent skin and mesoderm. It was found that either the mesoderm immediately underlying the sensory epithelium or

that left after its removal can form the capsule and concha of the middle chamber. The prospective vestibular skin can regenerate or replace the respiratory epithelium of the middle chamber but not the olfactory epithelium. The pit itself develops according to its prospective potencies.

8. Vestibular type skin in chorio-allantoic grafts differentiates several days sooner than in the normal controls. This acceleration of differentiation does not occur in grafts to a host embryo of the same age as the donor.

9. When olfactory epithelium differentiated in these grafts an olfactory nerve was formed which generally grew into forebrain tissue if any was present in the graft, whether or not prospective olfactory lobe material had been included. If brain tissue was not present or if the nerve failed to connect with brain the fibers either balled up near the epithelium or ran for a short distance into the mesoderm or along a cartilage bar. When brain tissue was not included in the grafts, frequently the nerve still pursued a course which with normal placode-brain relations would have brought it to the brain. This suggests that a mechanical factor directs its outgrowth rather than a continuously acting chemical or electrical stimulus emanating from the brain.

10. For purposes of comparison a few grafts of the olfactory placode with the skin and mesoderm adjacent to it were made between the limb buds of a 3-day embryo. The nasal complex developed well and differentiation in general resembled that found in the chorio-allantoic grafts. Olfactory nerves were formed which generally did not grow far from the epithelium but did in a few cases anastomose with the host nerves which entered the graft or grew from the graft into the host for a short distance.

LITERATURE CITED

ADELMANN, H. B. 1929 a Experimental studies on the development of the eye. I. The effect of removal of median and lateral areas of the anterior end of the urodele neural plate on the development of the eyes. J. Exp. Zool., vol. 54, pp. 249–290.

———— 1929 b Experimental studies on the development of the eye. II. The eye-forming potencies of the median portions of the urodelan neural plate. J. Exp. Zool., vol. 54, pp. 291–317.

———— 1934 A study of cyclopia in Amblystoma punctatum with special reference to the mesoderm. J. Exp. Zool., vol. 67, pp. 217–281.

BELL, E. T. 1906 Experimental studies on the development of the eye and the nasal cavities in frog embryos. Anat. Anz., Bd. 29, S. 185–194.

———— 1907 Some experiments on the development and regeneration of the eye and nasal organ in frog embryos. Roux' Arch., Bd. 23, S. 84–194.

BURR, H. S. 1916 The effects of removal of the nasal pits in Amblystoma embryos. J. Exp. Zool., vol. 20, pp. 27–58.

———— 1920 The transplantation of the cerebral hemispheres of Amblystoma. J. Exp. Zool., vol. 30, pp. 159–169.

—— 1924 Some experiments on the transplantation of the olfactory placode in Amblystoma. I. An experimentally produced aberrant cranial nerve. J. Comp. Neur., vol. 37, pp. 455–480.

BUTLER, E. 1935 The developmental capacity of regions of the unincubated chick blastoderm as tested in chorio-allantoic grafts. J. Exp. Zoöl., vol. 70, pp. 357–395.

CARPENTER, E. 1937 The head pattern in Amblystoma studied by vital staining and transplantation methods. J. Exp. Zool., vol. 75, pp. 103–129.

CLARKE, L. F. 1936 Regional differences in eye-forming capacity of the early chick blastoderm as studied in chorio-allantoic grafts. Phys. Zool., vol. 9, pp. 102–128.

COGHILL, G. E. 1924 Correlated anatomical and physiological studies of the growth of the nervous system of amphibia. IV. Rate of proliferation and differentiation in the central nervous system of Amblystoma. J. Comp. Neur., vol. 37, pp. 71–109.

COHN, F. 1903 Zur Entwicklungsgeschichte des Geruchorgans des Hühnchens. Arch. Mier. Anat., Bd. 61, S. 133–150.

DETWILER, S. R. 1927 Die Morphogenese des peripheren und zentralen Nervensystems der Amphibien im Licht experimentaller Forschungen. Naturwiss., Bd. 15, S. 873–895.

———— 1936 Neuroembryology. Macmillan. New York.

DISSE, J. 1897 Die erste Entwicklung des Riechnerven. Anat. Hefte., Bd. 9, S. 257–297.

GAUPP, E. 1906 Die Entwicklung des Kopfskelets. Hertwig's Handbuch der vergl. und exp. Entwicklungslehre der Wirbeltiere. Kap. 7.

HAMBURGER, V. 1933 A method of transplanting limb buds in chick embryos. Anat. Rec., vol. 55 (Suppl.), p. 58.

HIRAIWA, Y. K. 1927 Grafts of embryonic tissues of the rat on the chorio-allantoic membrane of the chick. I. Differentiation of ectodermal derivatives. J. Exp. Zool., vol. 49, pp. 441–457.

HOADLEY, L. 1924 The independent differentiation of isolated chick primordia in chorio-allantoic grafts. I. The eye, nasal region, otic region and mesencephalon. Biol. Bull., vol. 46, pp. 281–315.

HOLTFRETER, J. 1933 Einfluss von Wirtsalter und verschiedenen Organbezirken auf die Differenzierung von angelagertem Gastrula-Ectoderm. Roux' Arch., Bd. 127, S. 619–775.

———— 1936 Regionale Inductionen in xenoplastich zusammengesetzten Explantaten. Roux' Arch., Bd. 134, S. 466–550.

LANDACRE, F. L. 1921 The fate of the neural crest in the head of Urodeles. J. Comp. Neur., vol. 33, pp. 1–43.

LILLIE, F. R. 1908 The development of the chick. Henry Holt and Co., New York.

KNOUFF, R. A. 1935 The developmental pattern of the ectodermal placodes in Rana pipiens. J. Comp. Neur., vol. 62, pp. 17–73.

LEWIS, W. H. 1907 Experimental evidence in support of the theory of the outgrowth of the axis cylinder. Am. J. Anat., vol. 6, pp. 461–471.

LUNA, E. 1915 Ricerche sperimentali sulla morfologia dell'organo dell'olfatto negli anfibi. Arch. Ital. di Anat. e di Embriol., vol. 14, pp. 609–628.

MANGOLD, O. 1933 Isolationsversuche zur Analyse der Entwicklung bestimmter Kopforgane. Naturwiss., Bd. 21, S. 394–397.

MAY, R. M. 1927 Modifications des centres nerveux dues à la transplantation de l'oeil et de l'organ olfactif chez les embryons d'Anoures. Archives de Biol., vol. 37, pp. 337–363.

RAVEN, C. P. 1933 Zur Entwicklung der Ganglienleiste. III. Induktionsfähigkeit des Kopfganglienleisten-materials von Rana fusca. Roux' Arch., Bd. 13, S. 517–561.

RAWLES, M. E. 1936 A study in the localization of organ-forming areas in the chick blastoderm of the head process stage. J. Exp. Zool., vol. 72, pp. 271–315.

RÖHLICH, K. 1931 Gestaltungsbewegungen der präsumptiven Epidermis wahrend der Neurulation und Kopfbildung bei Triton taeniatus. Roux' Arch., Bd. 124, S. 66–81.

RUDNICK, D. 1932 Thyroid-forming potencies of the early chick blastoderm. J. Exp. Zool., vol. 62, pp. 287–312.

STONE, L. S. 1926 Further experiments on the extirpation and transplantation of mesectoderm in Amblystoma punctatum. J. Exp. Zool., vol. 44, pp. 95–132.

———— 1929 Experiments showing the role of migrating neural crest (mesectoderm) in the formation of head skeleton and loose connective tissues in Rana palustris. Roux' Arch., Bd. 118, S. 40–77.

WADDINGTON, C. H., AND A. COHEN 1936 Experiments on the development of the head of the chick embryo. J. Exp. Biol., vol. 13, pp. 219–235.

WEISS, P. 1934 In vitro experiments on the factors determining the course of the outgrowing nerve fiber. J. Exp. Zoöl., vol. 68, pp. 393–448.

WILLIER, B. H., 1924 The endocrine glands and the development of the chick. Am. J. Anat., vol. 33, pp. 67–103.

— 1933 Potencies of the gonad-forming area in the chick as tested in chorio-allantoic grafts. Roux' Arch., Bd. 130, S. 616–649.

PLATES

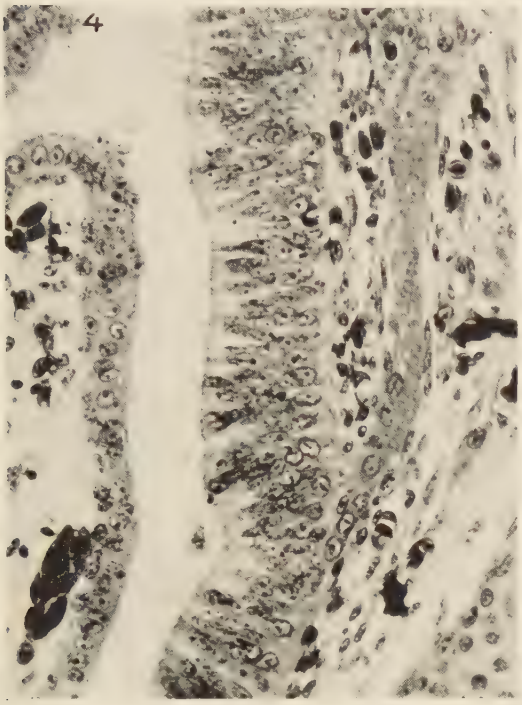
PLATE 1

EXPLANATION OF FIGURES

4 Photomicrograph of olfactory epithelium from a chorio-allantoic graft of the nasal region of a 27-somite embryo. Note the typical differentiation of the epithelium (description in text) and the olfactory cells migrating into the nerve. × 430.

5 Tubule of vestibular skin from a chorio-allantoic graft of the anterior part of the head, including all of the forebrain, of a 5-somite embryo. × 210.

6 Section through the nasal area of a chorio-allantoic graft of the tip of the head of a 12-somite embryo. V, vestibule; M, abnormal middle conch; N, nasal gland; O, olfactory epithelium. × 60.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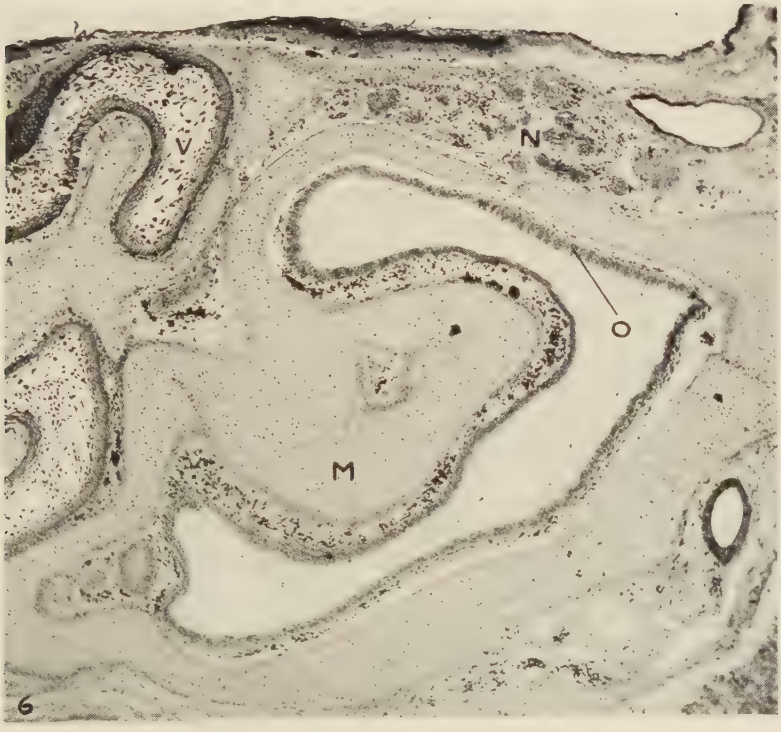
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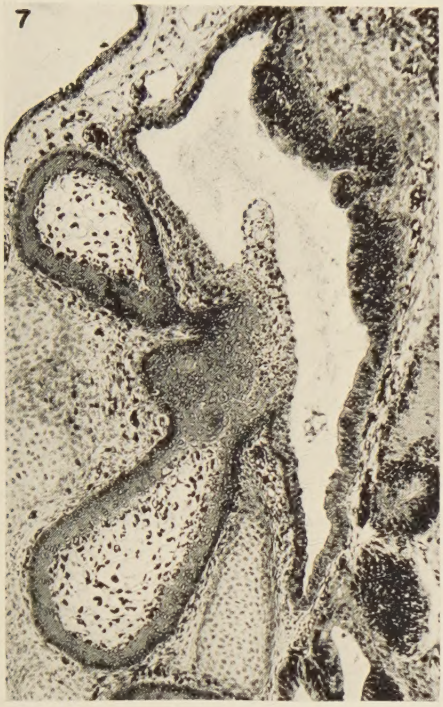
PLATE 2

EXPLANATION OF FIGURES

7 Section through the nasal area of a chorio-allantoic graft of the tip of the head of a 9-somite embryo showing olfactory and vestibular regions. × 100.

8 Middle chamber and conch from a chorio-allantoic graft of the left placode of a 42-somite embryo. Compare with figure 6. × 60.

9 Section through the vestibular region of a graft to the body wall of a 3-day host of both placodes of a 29-somite embryo. Total age of graft 10 days. Note that the differentiation of the vestibular skin is not accelerated. N, duct of nasal gland. × 60.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